Discovering System-Level Features of Biological Organization During Embryo Development

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Introduction

- Molecular biology has shown us that many key elements of cellular control reside at the genetic / genomic level

- DNA biochips (e.g. microarrays) provide important information regarding the molecular readout of cellular activities and regulation

- However, this is only part of the information that we need to understand the behavior of complex systems and disease processes …
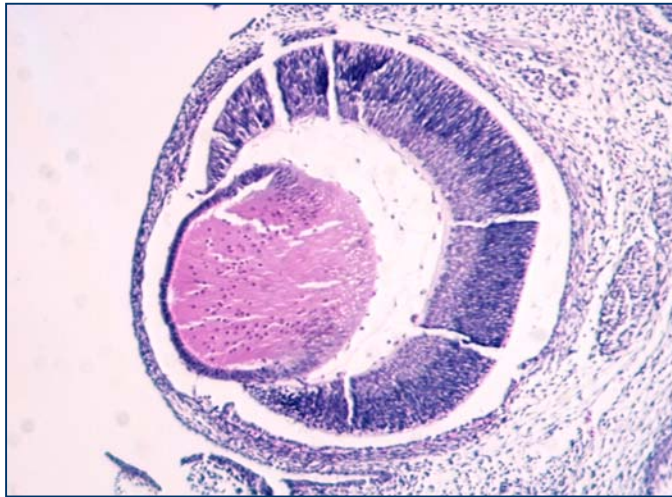
Digital Biology

- … we must build from existing knowledge of genetic and molecular function a more complete understanding of how a cell works (systems biology)

- Moving from molecular biology to modular biology – how do elements of structure fit into a modular hierarchy for control?

- And, given some members of the module can we use bioinformatics to complete the list and rely on computers to predict cellular behavior?
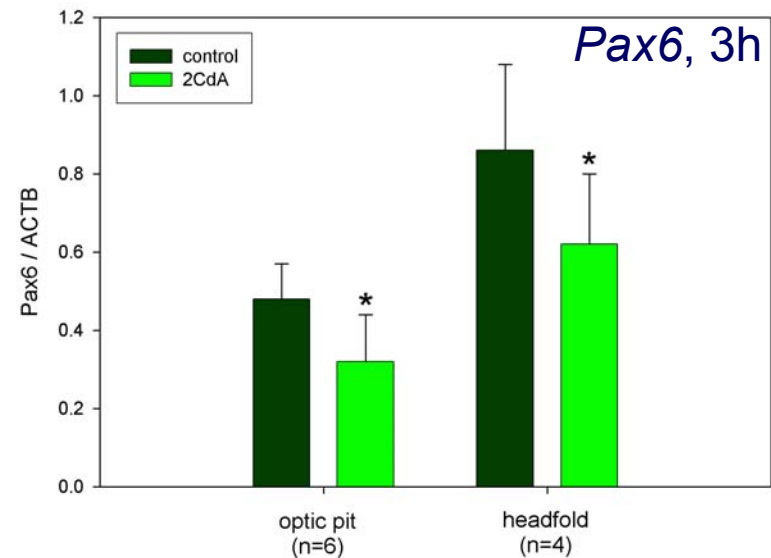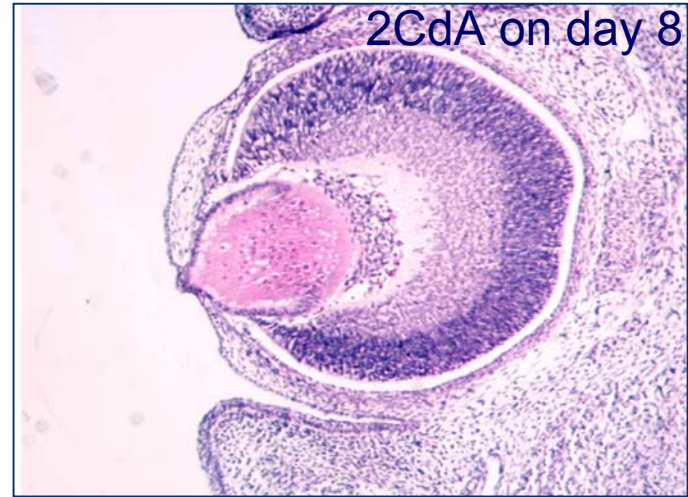
Peter's Anomaly
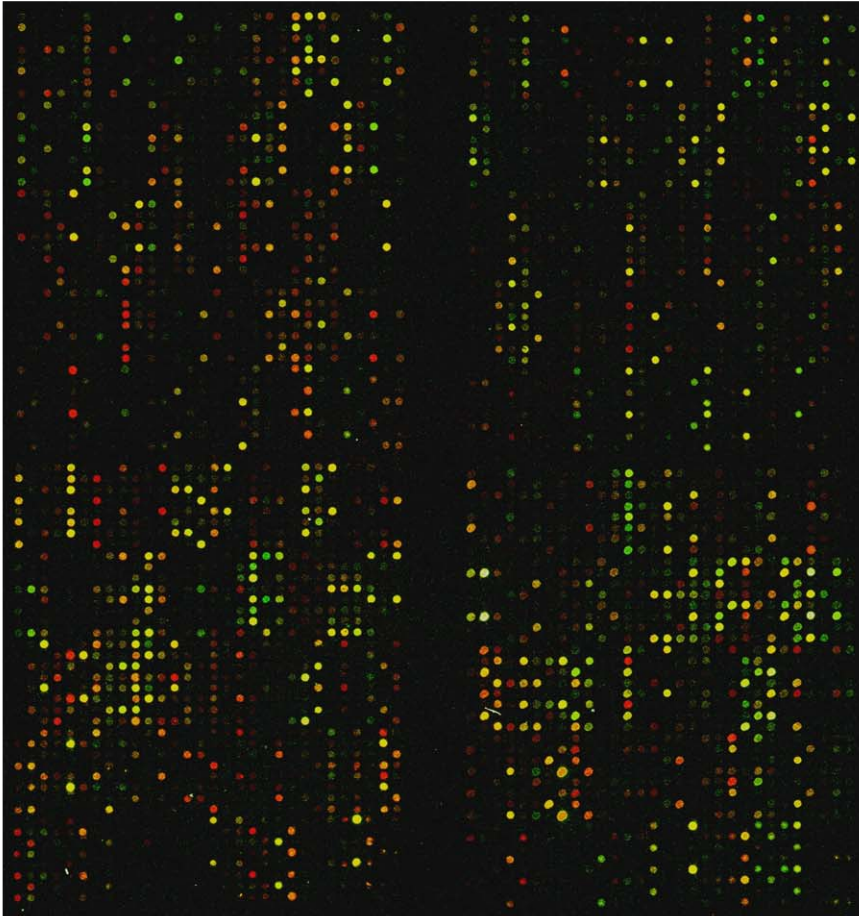## (keratolenticular dysgenesis)

day 14 mouse fetus
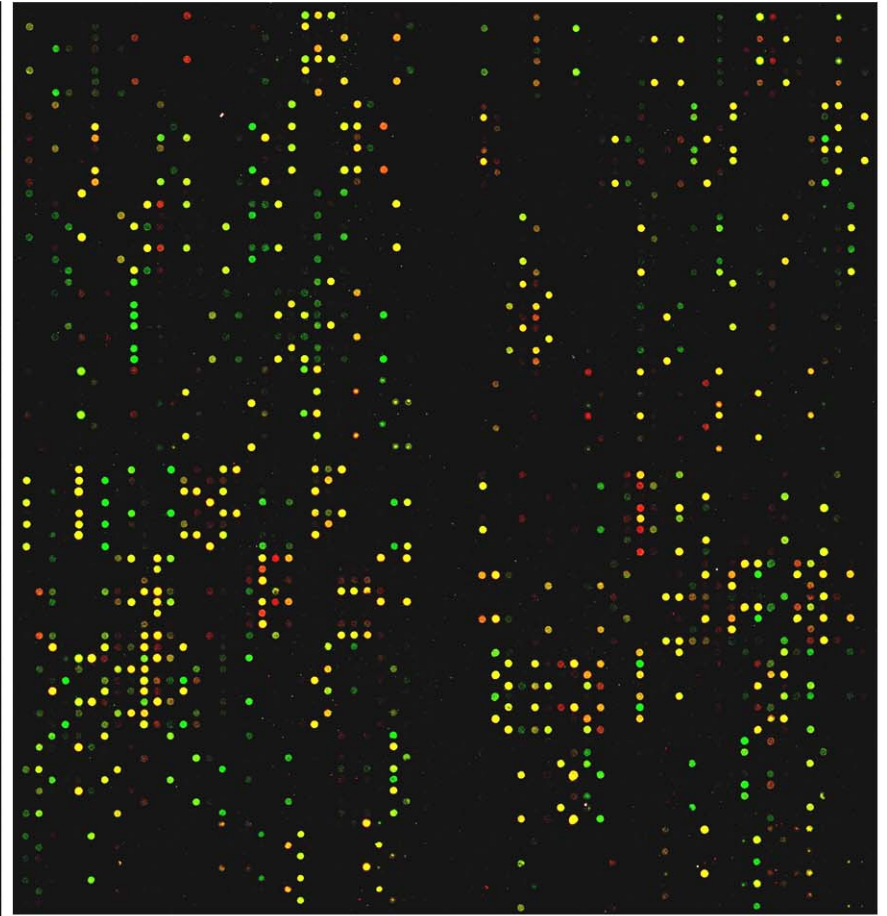
2CdA on day 8

Pax6, 3h

Early headfold stage embryos - EtOH

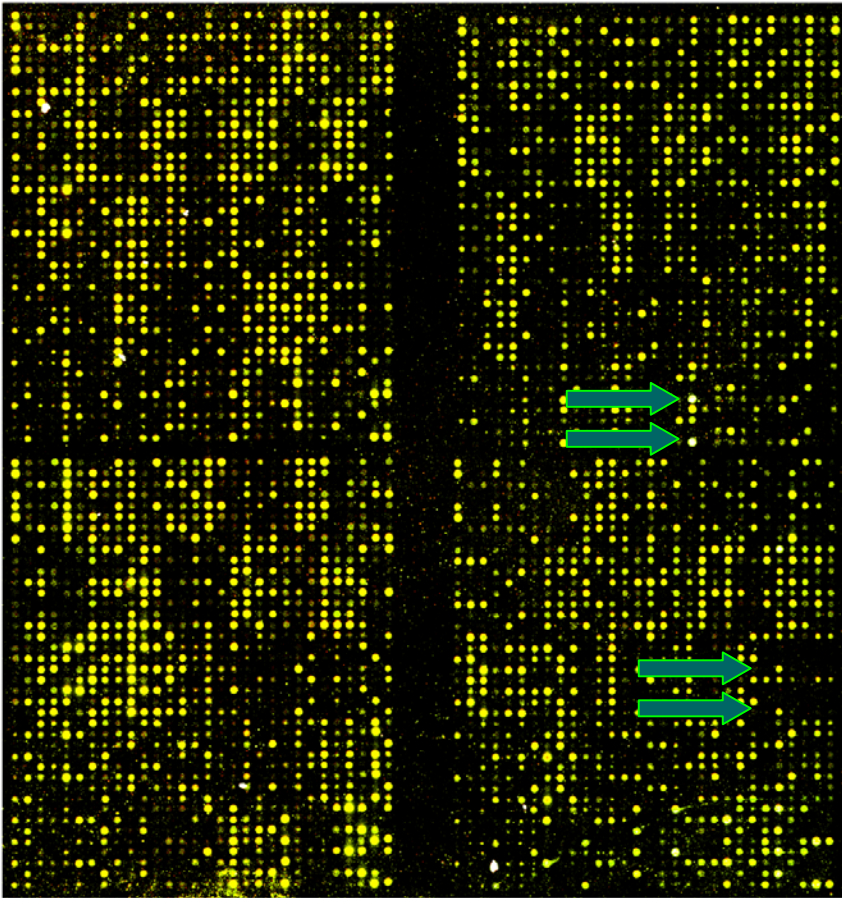control 6N - green / EtOH 6N - red

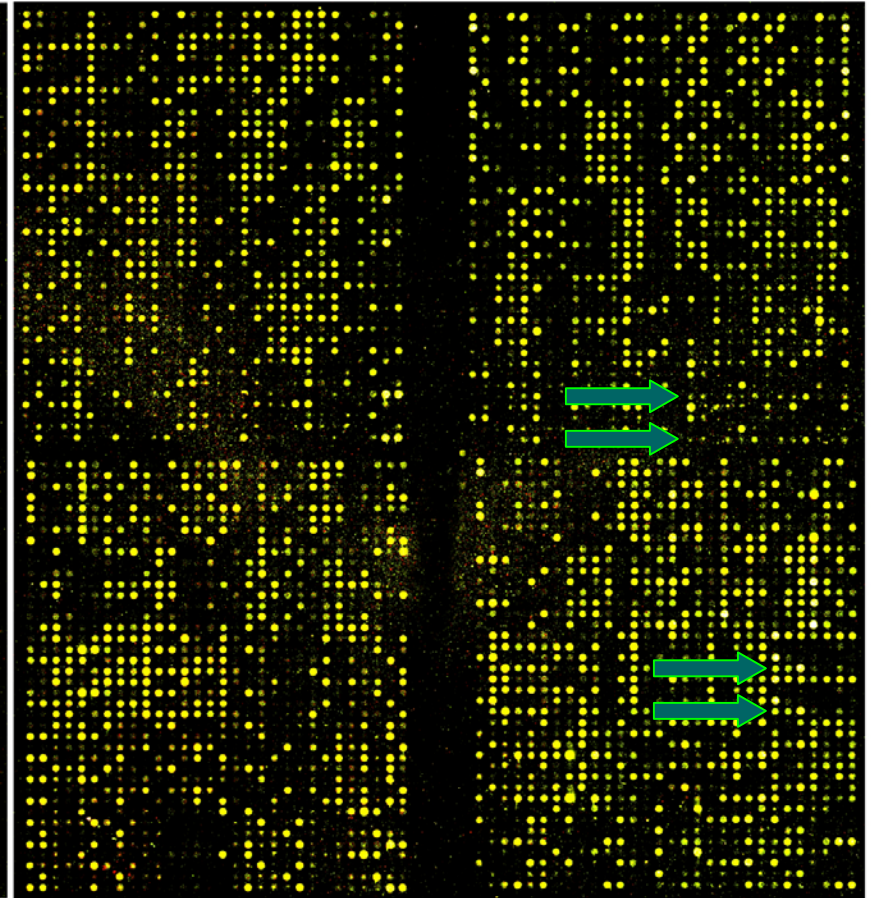EtOH 6N - green / control 6N - red

Early headfold stage embryos - species
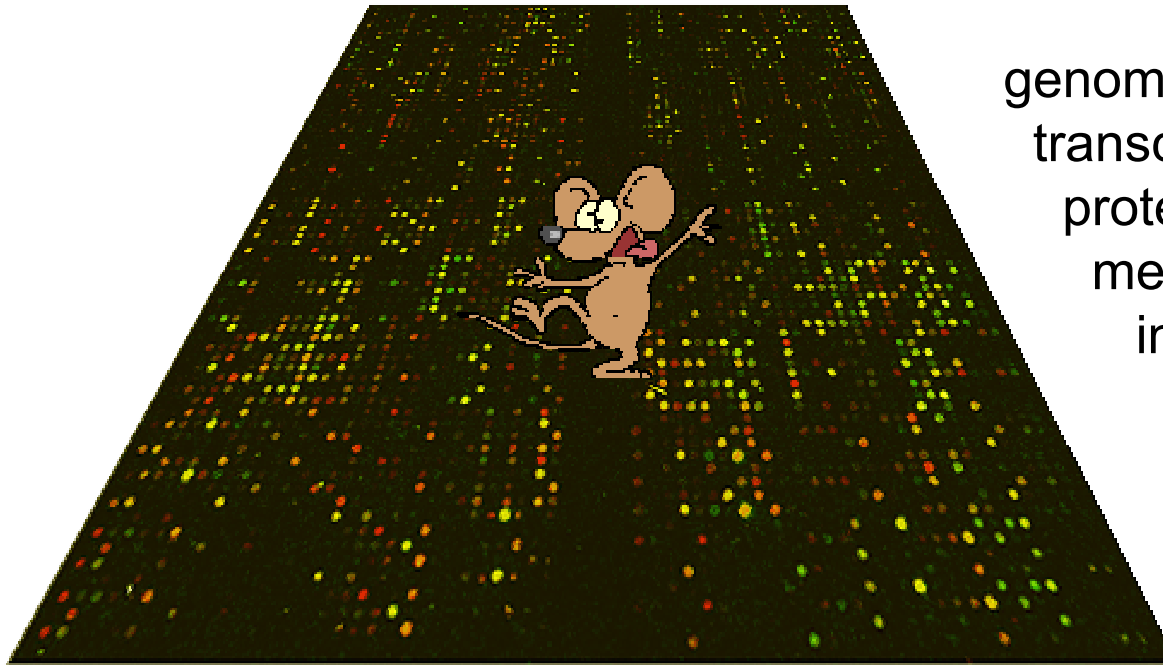
mouse embryo

rat embryo

Teratology Analyzer
A beginning

✳ 8 realms of digital biology:

genome
transcriptome
proteome
metabonome
interactome
cellunome
physiome
econome

Birth Defects Systems Manager
(BDSM)


- mid-level server for storage / retrieval of applications and data relevant to developmental biology and toxicity

- will house relational database built from digital information collected at all information levels, genome to phenotype

- "teratology analyzer" looks for systems-level features of the embryo during normal and abnormal development

What elements of system-level structure can we pick up with microarray analysis?

Working with early embryos we need signal amplification

- integrated chemistry platform from PerkinElmer Life Sciences works
- aRNA probably a good idea to enrich for precursor target cell populations


replica swap

FL-dCTP

BN-dCTP

Hybridize O/N at 55-59ºC

Tyramide Signal Amplification
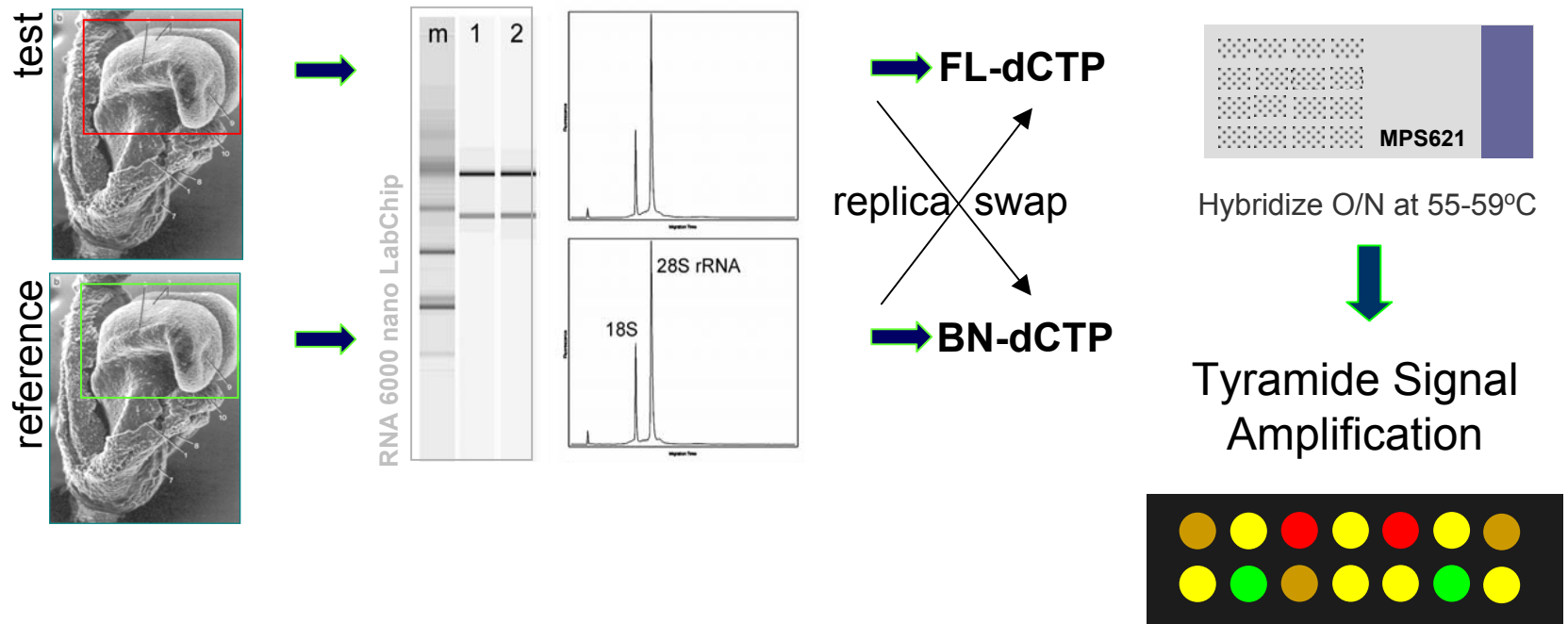
At least 280 genes varied consistently in ocular development for both *Mouse* and *Rat* embryos

http://www.med.unc.edu/embryo_images
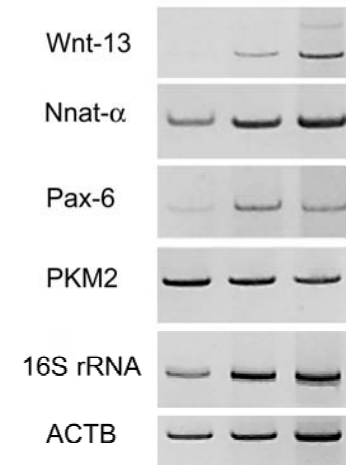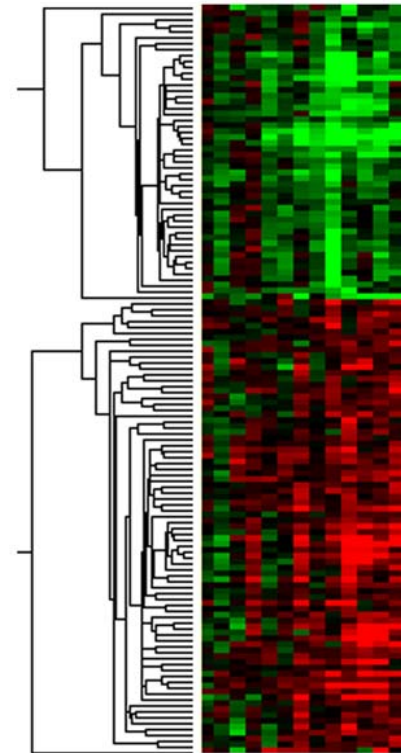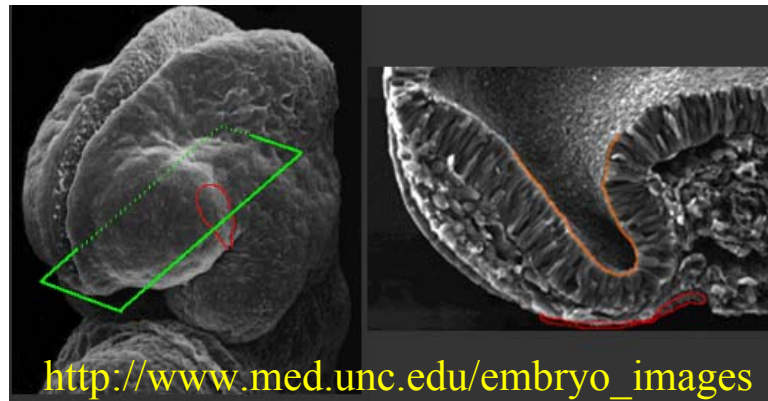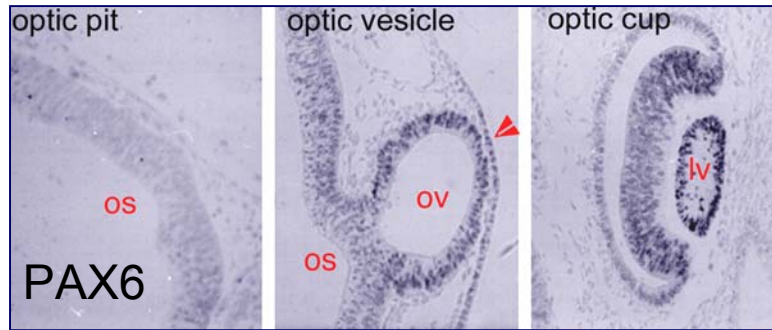
ribosomal proteins (10%)
glycolysis (9%)
HMG-1A (chromatin regulation)

calcium related processes (6%)
mitochondrion related processes (5%)
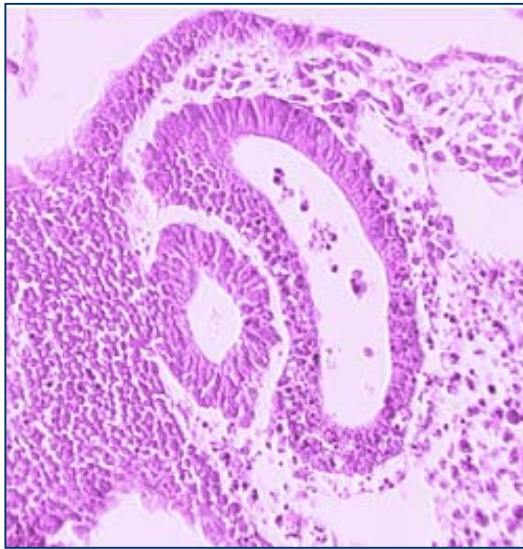actin cytoskeletal cycle (RTK pathway)

RT-PCR analysis
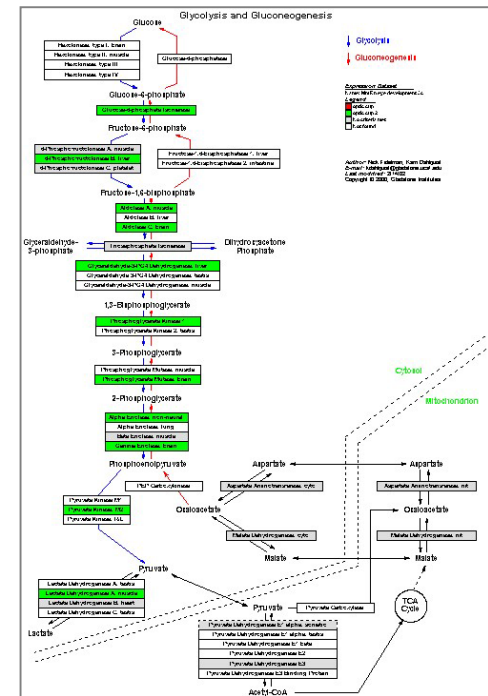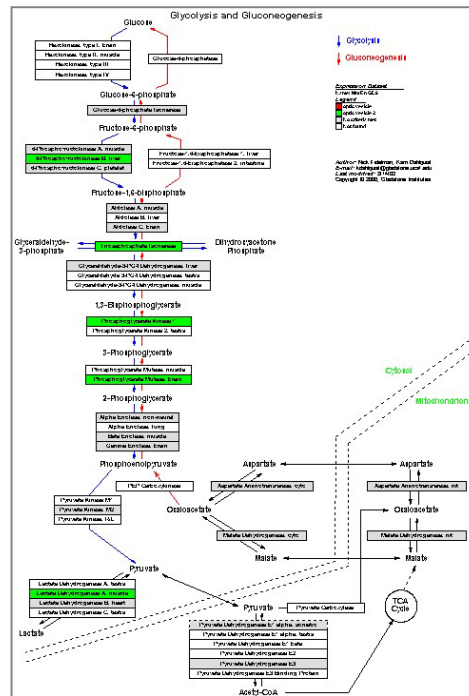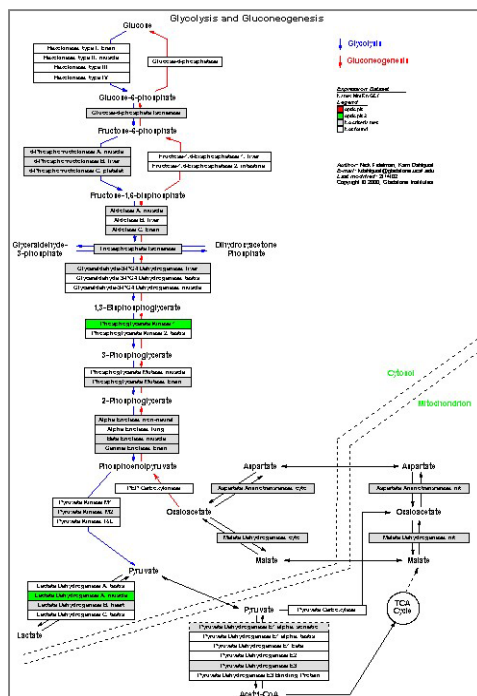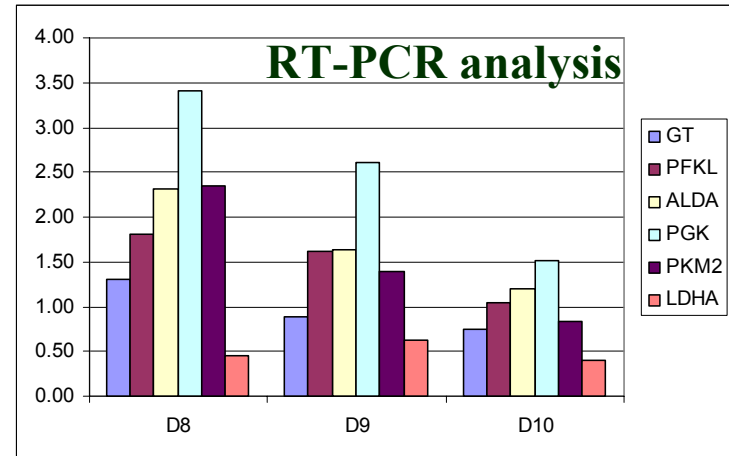
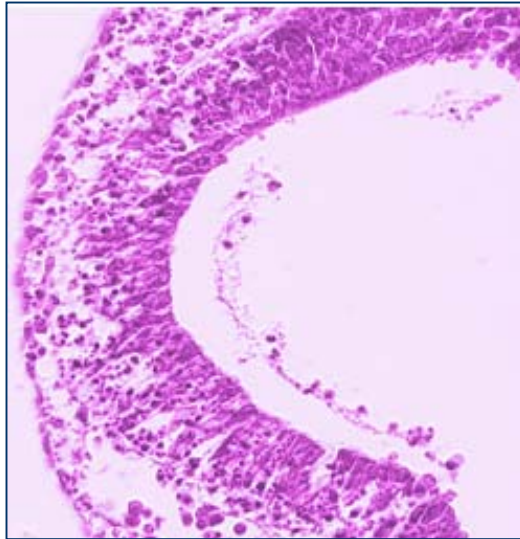optic pit

optic vesicle

optic cup

A mitochondrial connection?
hypoxia in whole embryo culture (day 9)

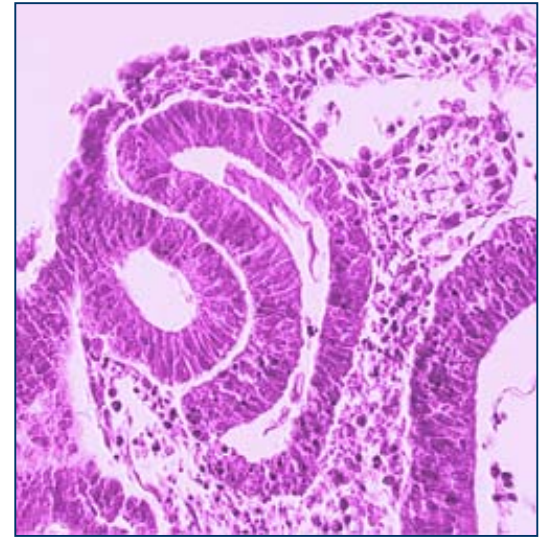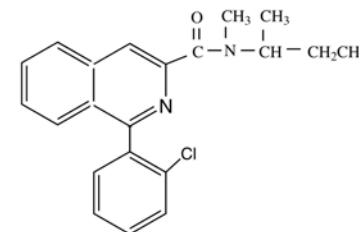| normoxia	hypoxia	+ PK11195
21% oxygen, 2h 21% oxygen, 16h	5% oxygen, 2h 21% oxygen, 16h	5% oxygen, 2h + PK11195 21% oxygen, 16h

- O'Hara et al. (2003) Reproductive Toxicology 17: 365-375

Intervention – PK11195

% embryos / fetuses malformed

agent	control	exposed	co-treated	n
2CdA 7.5 mg/kg	0.0% 0.0 – 0.0	50.2% ▲▲▲ 25.3 – 75.0	4.4% ▼▼▼ 0.0 – 11.9	7
MeHg 10 mg/kg	0.0% 0.0 – 0.0	47.7% ▲▲ 9.8 – 85.5	19.2% ▼▼▲ 1.7 – 36.7	6
Hypoxia 5%, 2 hr	0.0% 0.0 – 0.0	61.2% ▲▲▲ 47.8 – 75.6	24.6% ▼▼▲ 1.2 – 47.5	5

Mean incidence (95% C.I.); Newman-Keuls test versus control group (▲) or exposed group (▼)
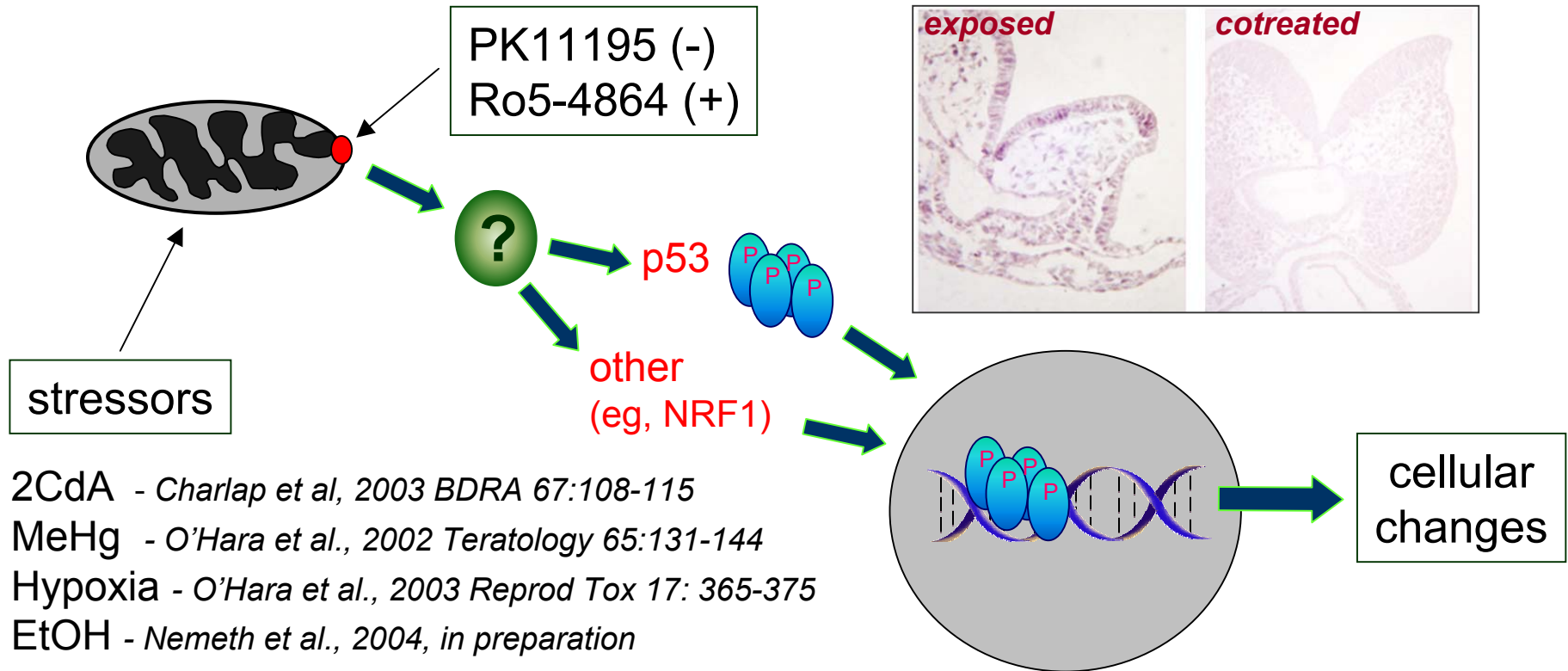
-O'Hara et al. (2002) Teratology 65:131-144
-Charlap et al. (2003) Birth Defects Research - A 67:108-115
-O'Hara et al. (2003) Reproductive Toxicology 17: 365-375

Mitochondrial benzodiazapine receptors

- 18 KDa protein (*Bzrp*) in outer mitochondrial membrane

- physiological roles in heme metabolism, cholesterol biosynthesis, oxygen homeostasis, and apoptosis

- essential for early mouse embryo development …
  … and much more

- PK11195, a high-affinity partial antagonist of the *Bzrp* has selective anti-teratogenic activity *in vivo* or *in vitro*

- *What is the mechanism?*

Working model: Bzrp checkpoint

PK11195 (-)
Ro5-4864 (+)

?

p53

other
(eg, NRF1)

stressors

exposed   *cotreated*

cellular
changes

2CdA - *Charlap et al, 2003 BDRA 67:108-115*
MeHg - *O'Hara et al., 2002 Teratology 65:131-144*
Hypoxia - *O'Hara et al., 2003 Reprod Tox 17: 365-375*
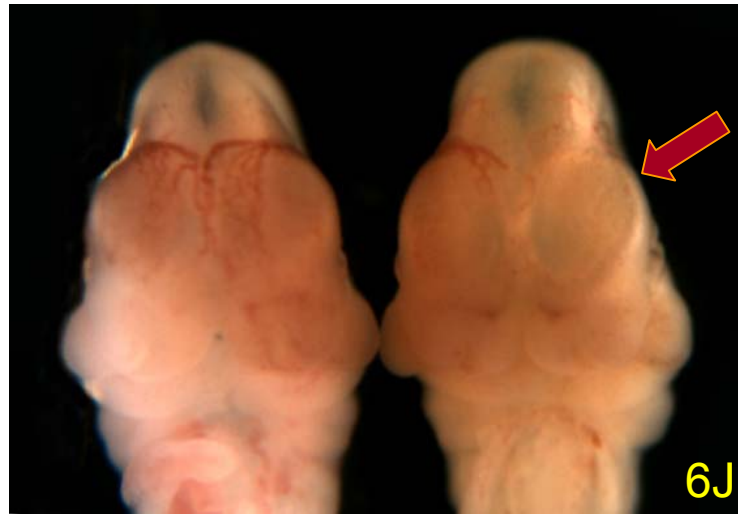EtOH - *Nemeth et al., 2004, in preparation*

1. Sensor – nature of the endogenous ligand evoked during exposure?
2. Transducer - how is Bzrp connected with key regulatory pathways?
3. Effector – downstream target genes in differential teratogenicity?

Alcohol-related malformations
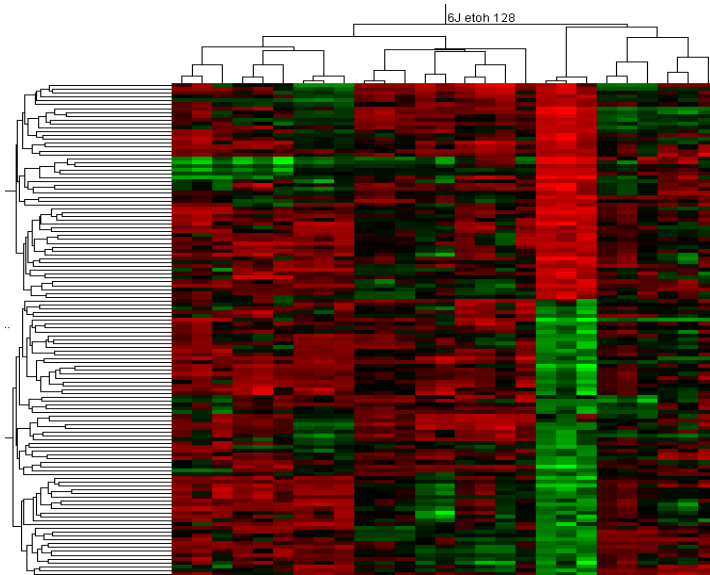## 72h after exposure to 2 x 2.9 g/kg EtOH on day 8

C57BL/6N
(insensitive)

6J
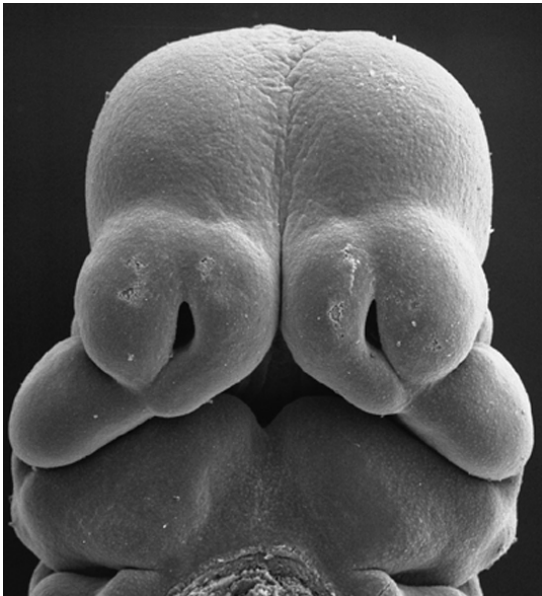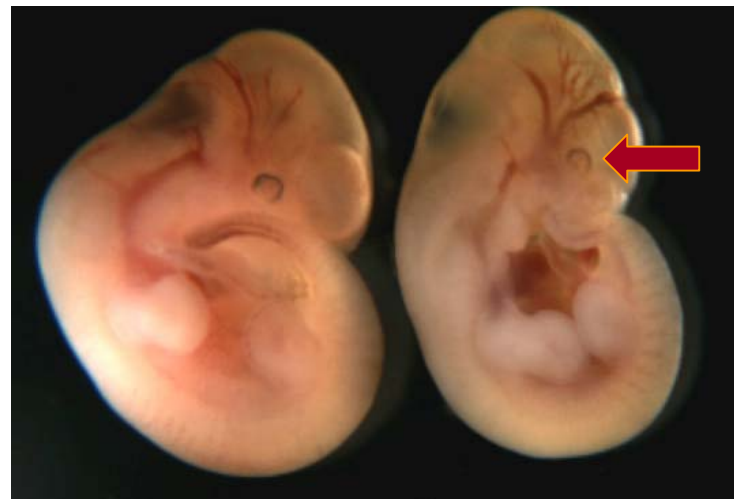
C57BL/6J
(sensitive)

Photos - Kathy Sulik UNC

Expression phenotype of the headfold
strains more (6J) or less (CD1, 6N) sensitive to EtOH exposure
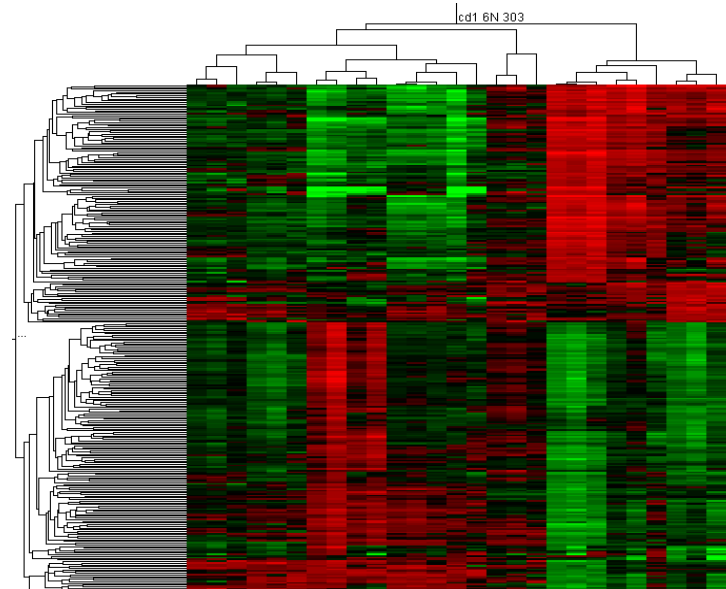

C57BL/6J

C57BL/6J

CD-1
C57BL/6N

57 genes
ASH1, CHROMOdomain (CHD3), CHROMObox (HP1)
CDP-alcohol PTrase, PI-4Kβ, PI transfer protein β
SCF6 creatine transporter, CK-B

72 genes
pancreatic proteins (lipase, protease, thread)
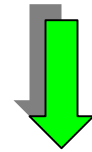PPase 1 (10), PPase 2 B56, PPase 3β
ZNF (-45, -151, -193, -SALL2)

144 genes
phosphofructokinase (PFKm, PFKl, PFK/FBP)
PTEN (2), v-akt (2), CSNK (I, II), CBP/p300
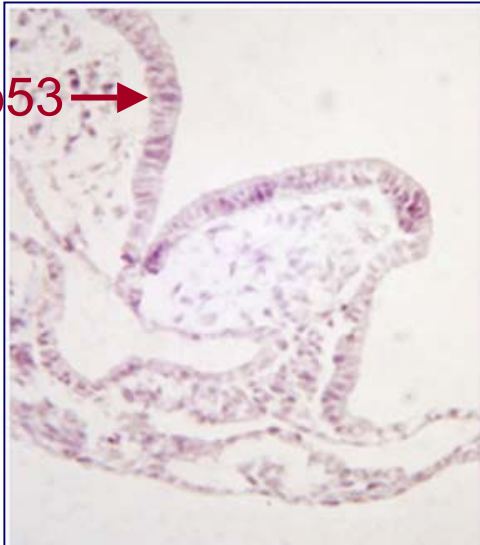HMG-1 AT-hook (2), SMARC-a2 and –a4 (BRG1), PUM-1

162 genes
HMG-box 1 (2), HAT, Sin3-HDAC1
cytosolic RPs (41)
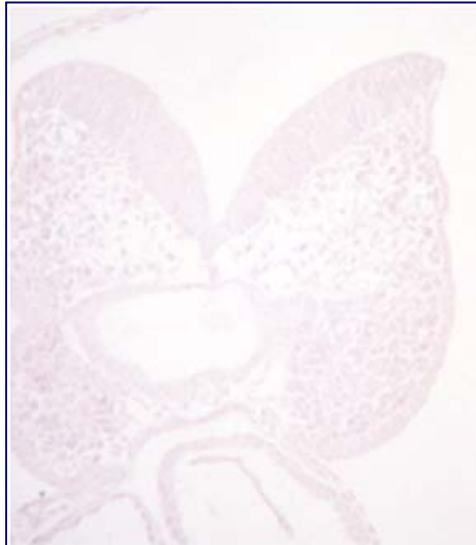Ras/Rab (7), 14-3-3 (2), thymosins (4)

p53 protein induction

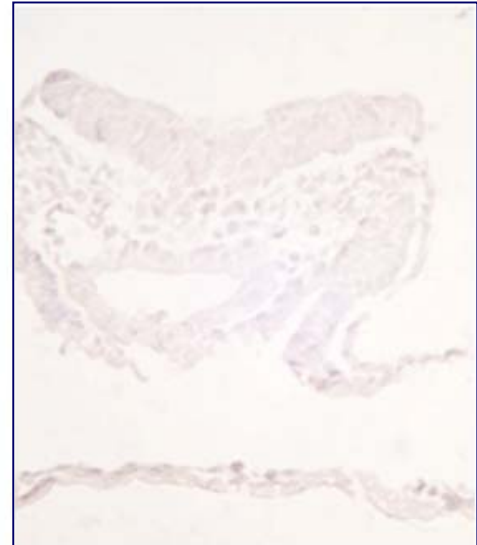*- Nemeth et al. 2004 (in preparation)*

| Rxt: | EtOH, 3h | EtOH + PK11195 | EtOH, 3h |
| Strain: | CD-1 | CD-1 | C57BL/6J |

p53 →

0% affected   0% affected (?)   ~20% affected (?)

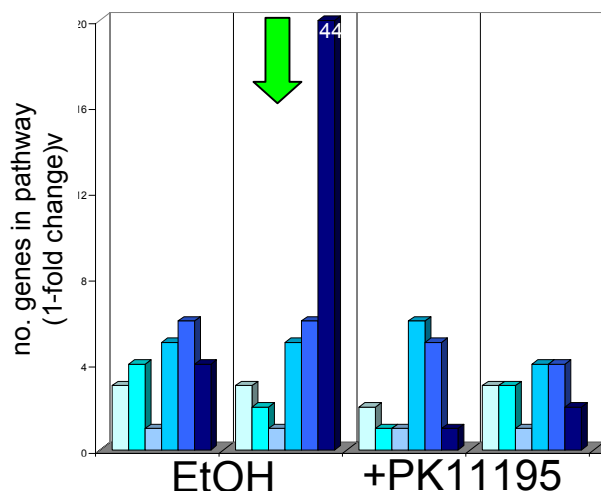C57BL/6J — CD-1 (outbred) — C57BL/6N

no. genes in pathway (1-fold change)

EtOH   +PK11195

Legend:
- ☐ Glycerolipid metabolism
- ☐ Glycolysis/Gluconeogenesis
- ☐ Inositol phosphate metabolism
- ☐ Oxidative phosphorylation
- ☐ Phosphatidylinositol signaling system
- ☐ Ribosomal proteins

http://david.niaid.nih.gov/David/

C57BL/6N C57BL/6J

CD-1 (outbred)
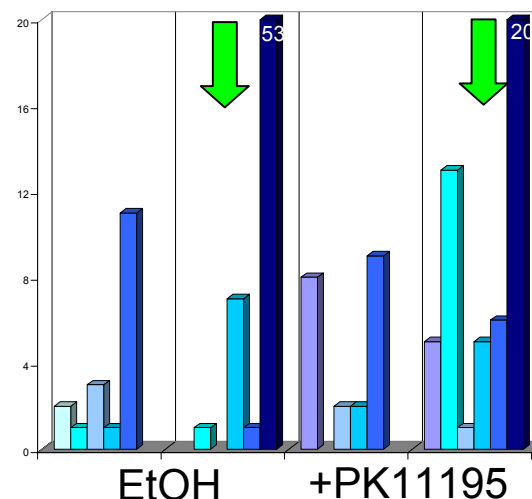
no. genes in pathway (1-fold change)v

EtOH   +PK11195

EtOH   +PK11195

☐ Glycerolipid metabolism
☐ Glycolysis/Gluconeogenesis
☐ Inositol phosphate metabolism
☐ Oxidative phosphorylation
☐ Phosphatidylinositol signaling system
☐ Ribosomal proteins

1794 → 363 → 151 → 114 →

experiments (K), alpha

all experiments K=50, P≤ 0.001

exposed, cotreated K=24, P≤ 0.05

PK11195, Ro5-4864 K=13, P≤ 0.05

p53-IR

2CdA
time course
(3.0, 4.5, 6.0 h)

EtOH
strain response
(6J, CD1, 6N)

MeHg
time course
(3.0, 4.5, 6.0

1794	→	140

experiments
(K), alpha

p53-IR
K=9, P$\leq$ 0.05

94

46

1794	→	150

experiments
(K), alpha

non-p53-IR
K=9, P$\leq$ 0.025

104

140   104
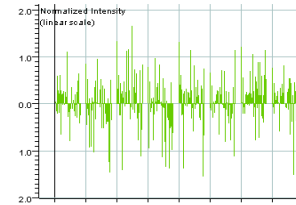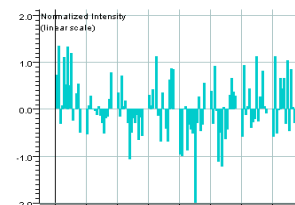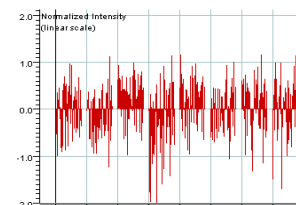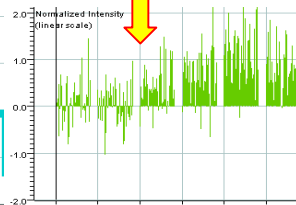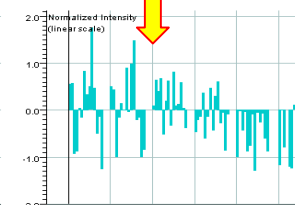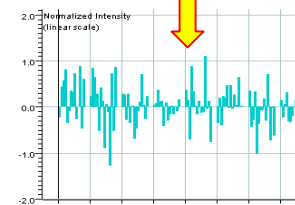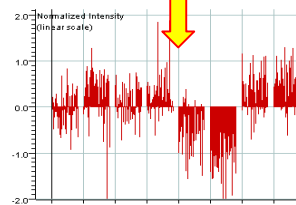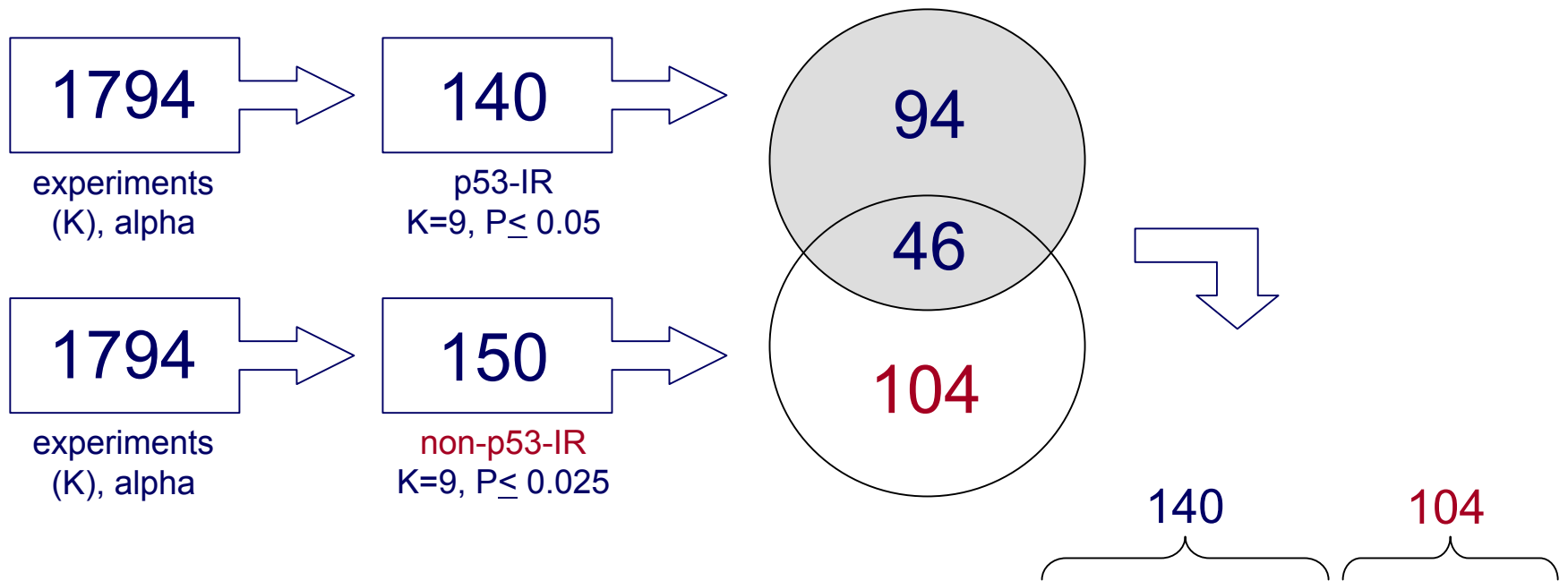
2CdA (3.0, 4.5, 6.0 h)
2CdA + Ro5-4864
EtOH (CD1, 6N)
EtOH + Ro5-4864 (CD1, 6N)
Ro5-4864 alone
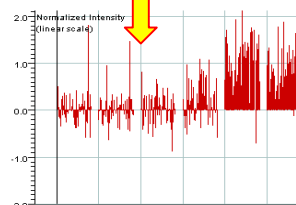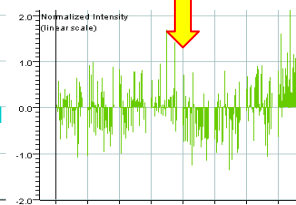
p53 protein induction
observed

2CdA + PK11195 (3.0, 4.5, 6.0 hr)
EtOH + PK11195 (6J, CD1, 6N)
EtOH (6J)
control, PK11195 alone

p53 protein induction
<u>not</u> evident

DRAGON:

Database Referencing Array Genes ONline
http://pevsnerlab.kennedykrieger.org/dragonview.htm

Chromatin Structure
SMARC A4, SMARC F1, SMARC C1, SMARC C2
HMGA1, HMGA1, HMGA1, HMG-17
NHP2, CHD-4, RAD21, ASH-1
p300/CBP interactor, DNMT1, MCM7

Signaling pathways
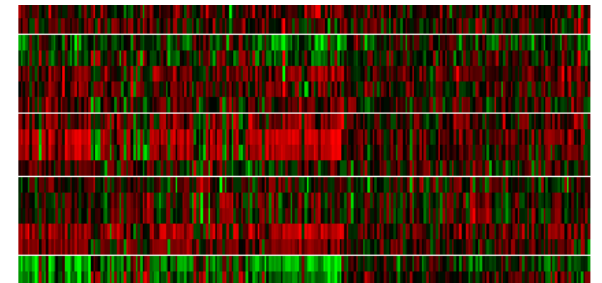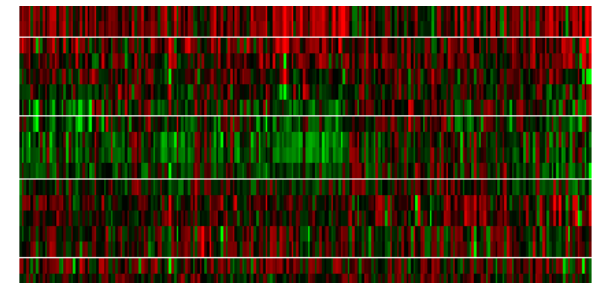14-3-3-beta/alpha, 14-3-3-zeta
Sgk1, p59ILK, TGFBR-II, Wnt-2b, IGF-BP2
v-akt1, v-akt2, PTEN, PTEN
PTPRF, PTPRG
LIM proteins CRP1, CRP2, and LH2
Ski, SkiP, EBHNA2
Cdk9, JAK1, N-WASP, macMARCKS
jagged-1, TCF-1
RhoGAP1, ARF2, Rsu-1, RasGAP1

potential upstream activators of p53
v-abl (via ATM)
CNK1 ε, CNK2 α1, CNK1γ2

Metabolic systems
PFKL, PFKm, PFKp
GSHR, GSHPx3, GSHPx3, GSHPx4, SOD3
LDHA, ENO1, ALDA, SDHD, ADH5, TPI, PKM2
SCF12.2, SCF12.4
peroxiredoxin 3, ATP citrate lyase
calcium channels A2, L, P/Q
ribophorin I, ribophorin II
FolBP
Hb-beta, Hb-alpha

Cellular structure
prothymosin alpha, prothymosin alpha
Col(VI)1, Col(VI)2, Col(I)alpha
ACTNA1, ACTNA2
ACTA1, ACTA2, ACTG1, ACTB, ACTBm
Filamin A, drebrin 1, DNM2
alpha-CP1, alpha-CP1
karyopherins B3, B2b, B1
EPH-B1, EPH-B1
FK506 BP8, FK506 BP12
PDI A4 and A6, CDC37

Summary and Conclusions

- ~10% of genes in the embryonic headfold were sensitive to low-level teratogen exposure (~10% average malformation rate)

- over half of the differentially regulated genes were clustered to experiments that show p53 protein induction by immunohistochimcal staining

- about 20% of the genes were further responsive to PK11195, which prevented p53 protein induction

Summary and Conclusions

- up-/down regulation of these genes fit a modular structure for synexpression (some genes go up or down but do so together)

- although synexpression groups may have a connection to p53 protein induction the directionality (up or down) was quite varied between agents

- this directionality was more consistent with the inherent susceptibility of embryos -- clearly, gene expression data is only part of the information needed to understand the complex systems

Acknowledgements